Journal of Medical Virology 59:164–168 (1999)

Seroprevalence of Antibodies to Hepatitis E Virus in the Normal Blood Donor Population and Two Aboriginal Communities in Malaysia

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The prevalence of antibodies to hepatitis E virus (HEV) has been examined in many countries, but such studies have generally been limited to majority populations such as those represented in healthy blood donors or cross sections of urban populations. Due to its major route of enteric transmission, large differences in HEV prevalence might be expected between populations in the same country but with different living conditions. Using an ELISA based on GST-ORF2.1 antigen, the prevalence of IgG-class antibodies to HEV was examined in three distinct populations in Malaysia: the normal (urban) blood donor population and two aboriginal communities located at Betau, Pahang and Parit Tanjung, Perak. IgG anti-HEV was detected in 45 (44%) of 102 samples from Betau and 15 (50%) of 30 samples from Parit Tanjung, compared to only 2 (2%) of 100 normal blood donors. The distribution of sample ELISA reactivities was also consistent with ongoing sporadic infection in the aboriginal communities, while there was no significant relationship between HEV exposure and age, sex, or malaria infection. The high prevalence of antibodies to HEV in the two aboriginal communities indicates that this group of people are at high risk of exposure to HEV compared to the general blood donors, and the results suggest that studies of HEV seroprevalence within countries must take into account the possibility of widely varying infection rates between populations with marked differences in living conditions. ***J. Med. Virol. 59:164–168, 1999.***

KEY WORDS: hepatitis E antibodies; aborigines; Malaysia; seroprevalence

INTRODUCTION

Hepatitis E virus (HEV) is a single-stranded RNA virus that is transmitted by the faecal–oral route, with some similarities to members of the Caliciviridae family. HEV has been responsible for outbreaks of waterborne hepatitis in many developing countries, including the Indian subcontinent, Central Asia, Africa, and Central America where environmental sanitation facilities are inadequate [reviewed in Krawcynski et al., 1996]. Travellers to endemic areas are at major risk of HEV infection [Dawson et al., 1992], but sporadic cases of acute hepatitis E without an implicated travel history have also been reported in Europe [Jardi et al., 1993; Zaaijer et al., 1993], Australia [Heath et al., 1995], and the United States [Kwo et al., 1997].

HEV generally causes self-limiting acute hepatitis, similar to hepatitis A virus (HAV), but differs from HAV in that it can cause fulminant hepatitis in pregnant woman where the mortality rate can be as high as 20% [Khuroo et al., 1981]. There is currently no HEV vaccine available commercially; however, promising candidate vaccines have been reported [Tsarev et al., 1994; Yarbough et al., 1996], and there is every reason to believe that active immunisation could be useful in the control of HEV. However, appropriate use of such vaccines would require a much better understanding of the incidence of HEV infection worldwide. In areas with endemic rates of viral hepatitis the relative contribution of HAV and HEV to clinical cases varies widely. In China, almost 50% of cases are caused by HAV with lower rates (estimated at about 10%) due to HEV. In Taiwan, 29.5% of cases are caused by HAV and <1% was due to HEV. In contrast, in the Indian subcontinent, 50% of cases of viral hepatitis are caused by HEV and approximately 15% is due to HAV [Tandon and Tandon, 1996]. Thus the importance of HEV in endemic areas cannot be guessed, and must be measured specifically.

Grant sponsors: Department of Medical Sciences, Faculty of Medicine and Health Science, University Putra Malaysia

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Accepted 19 March 1999

To our knowledge, the seroprevalence rate of anti-HEV in Malaysia has not been examined. One study in a neighbouring country, Singapore, showed that hepatitis E IgG was detected in 3 of 25 (12%) patients with acute non-A, non-B, non-C hepatitis, 2 of 20 (10%) with cirrhosis, 3 of 10 (30%) with hepatocellular carcinoma and 13 of 79 (16%) of patients with fatty liver, while in a random adult population over the age of 20 years, anti-HEV IgG was detected in 3 of 87 (3.4%) individuals [Oon et al., 1992]. However, the prevalence of HEV in a highly urbanised population such as Singapore might not reflect the true prevalence of HEV in the region.

The seroprevalence rate of hepatitis E antibodies was determined in the normal (urban) blood donor population in Malaysia and, for comparison, two aboriginal communities who might be at relatively high risk of hepatitis E infection. The aboriginal population in peninsular Malaysia are known as the Orang Asli, and they comprise only 0.5% of the total population of 95,529 people. Some of the Orang Asli settlements are in forest-fringed villages, while others live in deep jungles. They are exposed to many diseases such as tuberculosis, cholera, typhoid, and malaria, and they constitute approximately 74% of total malaria infection in Malaysia. Compared with modern trends of nutritional health, the Orang Asli do not fare well. This could be explained by their dietary habits and living environment whereby they are often without clean water supply and proper sewage disposal [Tengku Ariff et al., 1997]. Of 179 households surveyed, 134 used water supply from the gravity feed system while the rest used river water for washing, cooking and drinking. Only 63.6% of families boiled their water before drinking.

The results indicate that HEV infection is very common among the aboriginal population in Malaysia, and stress the need for studies of HEV prevalence in whole communities rather than easily accessible populations such as blood donors.

MATERIALS AND METHODS

Sera

In December 1990, 102 serum samples from Betau, Pahang in Central Malaysia and 30 samples from Parit Tanjung, Perak in North Malaysia were obtained and stored at −20°C until use. The sera were collected previously for a malarial matrix survey and tested for *Plasmodium falciprum* and *Plasmodium vivax*. In 1998, 100 samples collected from blood donors were obtained from the Blood Services Centre, Hospital Kuala Lumpur after routine blood screening.

Detection of Anti-HEV

IgG anti-HEV was detected by ELISA using the recombinant antigen glutathione S-transferase (GST)-ORF 2.1 [Li et al., 1994]. The ORF 2.1 fragment comprises the carboxy-terminal 267 amino acids of the HEV capsid (ORF2) protein and presents a conformational epitope, which allows optimal detection of convalescent phase sera [Li et al., 1997]. The IgG ELISA was carried out as described in detail elsewhere [Anderson et al., 1999], and all assay reagents (including antigen-coated plates) were provided by AMRAD Biotech (Melbourne, Australia). The ORF2.1 ELISA allows for detection and quantitation of both acute and convalescent phase anti-HEV IgG [Anderson et al., 1999]. For the purpose of comparative studies, International reference standard HEV serum 95/584 (a gift from Morag Ferguson, NIBSC, UK) was tested in duplicate at dilutions from 1:200 to 1:12,800, and the cutoff for the IgG ELISA is set at 0.9 × the assay OD for the International reference standard dilution of 1:6,400 (15.6 mIU/ml), thus representing a cutoff of approximately 14 mIU/ml in this study.

TABLE I. Prevalence of Anti-HEV in Healthy Blood Donors and Two Orang Asli Communities

	n	Positive (%)
Blood donors	100	2 (2)
Orang Asli (Betau)	102	45 (44)
Orang Asli (Parit Tg.)	30	15 (50)

Serum specimens, positive control, and negative control were diluted 1:300 with specimen diluent. After the addition of 100 μl of each diluted specimen and controls to the wells, the plate was incubated for 60 minutes at room temperature. The wells were then washed 4 to 6 times using 1× wash buffer, inverted and tapped firmly onto absorbent paper towels. Horseradish peroxidase-conjugated sheep anti-human IgG (100 μl, 1:100 dilution in conjugate diluent) was added to each well and incubated for 60 minutes at room temperature followed by washing as before and addition of 100 μl of TMB substrate. After 20–30 minutes, 100 μl of stop solution was added to each well. The absorbance was read using a 450-nm filter with 615–620 nm reference filter. Samples giving positive results on initial testing were retested in duplicate, and only those in which both duplicate wells were positive were considered as confirmed positive.

Statistical Analysis

Frequencies of HEV IgG reactivity between groups were compared using the χ^2 test (Statistical Package for Social Science 8.0). $P < 0.05$ was considered significant.

RESULTS

Of the 102 samples from Betau and 30 from Parit Tanjong that were tested for HEV-specific IgG, 45 samples (44%) from Betau were positive for the antibody, while 15 (50%) of 30 samples from Parit Tanjung were found to be positive for anti-HEV (Table I). The prevalence rates of anti-HEV in Orang Asli from Betau and Parit Tanjung of 44 and 50%, respectively, were much higher than normal blood donors from Kuala Lumpur in which the prevalence was only 2% (Table I).

TABLE II. Prevalence of Anti-HEV in Orang Asli and Blood Donors According to Age

Age group	Orang Asli (Betau)			Orang Asli (Parit Tanjung)			Blood donors		
	n	Pos	%	*n*	Pos	%	*n*	Pos	%
1–10	52	21	40	0	—	—	0	—	—
11–20	20	10	50	4	2	50	4	0	0
21–30	14	6	43	5	2	40	53	0	0
31–40	6	4	67	7	1	14	31	1	3
41–50	7	3	43	4	2	50	11	1	9
51–60	3	1	33	6	4	66	1	0	0
61–70	0	—	—	3	3	100	0	—	—
71–80	0	—	—	1	1	100	0	—	—
Total	102	45	44	30	15	50	100	2	2

Within the positive samples from Betau, 17 were obtained from males and 28 from females, but this difference was not significant (data not shown). There was also no significant association of HEV reactivity and increasing age (Table II), but the very small numbers from groups older than 20 makes this comparison difficult. It appears, however, that exposure to HEV occurs at an early age in these communities, which would be expected to result in a much higher prevalence among older individuals. This study did not include sufficient older individuals to make a firm conclusion on this point.

Frequency analysis of anti-HEV IgG reactivity expressed as the ratio of sample to cutoff (S/CO) showed that among 100 normal donor sera, the two positive samples had weak reactivity with S/CO values of 1.24 and 2.4 (Fig. 1), with the negative samples predominantly in the range of <0.5 S/CO. Of the 45 positive samples from the Orang Asli community at Betau, 25 samples (56%) were found to be weakly reactive ($1 \leq$ S/CO range < 2), 3 (7%) of intermediate reactivity ($2 \leq$ S/CO < 2.5) and 17 (37%) had high reactivity with S/CO > 2.5 (Fig. 1). Positive sera from Parit Tanjung had a similar distribution of reactivities (Fig. 1); however, the total number of such samples (15) is not suitable for this analysis.

The data were analysed using the Statistical Packages for Social Sciences (SPSS Version 8.0 for Windows). It was found that there is no significance relationship between age, sex, presence of malaria infection, and hepatitis E antibodies ($P > .05$) (data not shown).

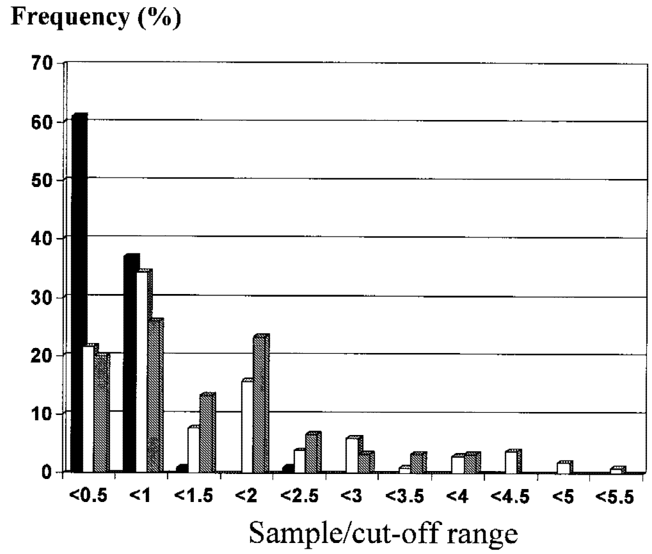

Fig. 1. Frequency distribution of serum IgG anti-HEV reactivity in different populations. (■) Normal donor; (□) sera from Betau; (▧) sera from Parit Tanjung. The ratio of sample OD to cutoff was determined for each sample.

DISCUSSION

One striking feature of the epidemiology of HEV infection is that even in populations with rates of HAV exposure approaching 100%, the rate of detection of IgG anti-HEV is rarely greater than 50% in any age group. In addition, HEV infection appears to have a much more restricted geographical range than that of HAV, with relatively low rates of HEV exposure reported in South American countries, for example. In countries that are considered endemic for both HAV and HEV, their relative importance as a cause of clinical hepatitis also varies widely. These factors argue for a close examination of HEV seroprevalence in specific populations, rather than extrapolation from the incidence of other enteric infections.

The epidemiology of hepatitis A infection has been studied more widely compared to that of hepatitis E, at least partly because of the lack of serological assays suitable for the detection of past HEV infection. Wide variability in the sensitivity of assays has been reported [Mast et al., 1998], while some sensitive assays (such as those based on baculovirus-expressed subviral particles) have shown high levels of reactivity with sera from presumably nonendemic areas, such as the United States [Thomas et al., 1997], raising doubts about their specificity. Conversely, the ELISA based on ORF2.1 antigen appears to be both sensitive and specific, showing a clear differential between HEV prevalence in endemic and nonendemic areas (Nepal and Australia, respectively) [Anderson et al., 1999]. This has allowed us to determine the prevalence of hepatitis E antibodies in Malaysia, in which we also wished to examine the possible difference between a high-risk group, that is, the aboriginal people who live in conditions of inadequate environmental hygiene and sewage disposal, as well as poor quality drinking water, compared to the general population in the urban areas.

The results demonstrate clearly that, within a single country, rates of exposure to HEV can vary greatly, from 2% in urban areas to 44–50% in aboriginal communities. This has clear implications for further studies of HEV epidemiology and eventual control by vaccine. The HEV seroprevalence of 2% in the Malaysian normal blood donor population is comparable to that reported in The Netherlands [Mushahwar et al., 1996] and Australia [Anderson et al., 1999]. The high positivity rates and the distribution of reactivities (by

S/CO) in the two Orang Asli communities were similar to those of residents of the Kathmandu Valley, Nepal using the same ELISA kit [Anderson et al., 1999] and contrast with the uniformly high S/CO ratios observed for epidemic sera [Anderson et al., 1999], and therefore appear to be consistent with ongoing, sporadic exposure to HEV, as seen in Nepal [Clayson et al., 1997].

Although no significant association of HEV prevalence was observed with age in this study, it was notable that the age distribution of subjects was very uneven, with most of the sera from Betau obtained from young children. Not surprisingly, comparison of healthy blood donors and Orang Asli from Betau of the same age group (21–40) still revealed the same difference in anti-HEV rates, from 2% in donors to 50% (n = 20) in Orang Asli from Betau. Further studies are required to clarify the age relationship of exposure to HEV in these communities, which may give clues to possible control mechanisms.

It is concluded that the two Orang Asli communities studied have very high rates of exposure to HEV, in stark contrast to urban residents in Malaysia. Perhaps, this is not surprising due to the marked difference in the living conditions of the two groups. The major transmission route of HEV is by contaminated water. Most of the aboriginal population do not have adequate environmental sanitation facilities; they use water from the rivers for washing and drinking purposes as well as for their toilet facility. In addition, consumption of undercooked food and unboiled drinking water associated with Orang Asli traditions or habits would lead to increased risk of infection by HEV. However, the possible role of animals, including rodents, pigs, and monkeys as possible reservoirs for HEV also deserves further study. In addition to many primates, rats and pigs have been shown to be susceptible to experimental infection with human HEV strains [Balayan et al., 1990; Karetnyi et al., 1993; Maneerat et al., 1996; Meng et al., 1998]. The recent discovery of a swine strain of HEV [Meng et al., 1997] and the detection of at least two patients infected with a similar strain in the United States [Schlauder et al., 1998] has greatly supported the possibility of zoonotic spread of HEV in nonendemic areas of human HEV. In addition to these concerns, the finding that high rates of HEV exposure can be maintained in countries with otherwise low rates of endemicity should be considered in planning the control of HEV.

ACKNOWLEDGMENTS

We wish to thank Dr. Yasmin Ayob, the Director of Blood Services Centre, Hospital Kuala Lumpur for providing serum samples from the normal donor population, and AMRAD Biotech (Melbourne, Australia) for the provision of HEV IgG ELISA kits.

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